

# Identification of Historic Apple Trees in the Southwestern United States and Implications for Conservation

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**Abstract.** Many apple varieties commonly planted in the United States a century ago can no longer be found in today's orchards and nurseries. Abandoned farmsteads and historic orchards harbor considerable agrobiodiversity, but the extent and location of that diversity is poorly understood. We assessed the genetic diversity of 280 apple (*Malus × domestica* Borkh.) trees growing in 43 historic farmstead and orchard sites in Arizona, Utah, and New Mexico using seven microsatellite markers. We compared the samples to 109 cultivars likely introduced into the southwest in the late 19th and early 20th centuries. Genetic analysis revealed 144 genotypes represented in the 280 field samples. We identified 34 of these 144 genotypes as cultivars brought to the region by Stark Brothers Nursery and by USDA agricultural experiment stations. One hundred twenty of the total samples (43%) had DNA fingerprints that suggested they were representative of these 34 cultivars. The remaining 160 samples—representing 110 genotypes—had unique fingerprints that did not match any of the fingerprinted cultivars. The results of this study confirm for the first time that a high diversity of historic apple genotypes remain in homestead orchards in the U.S. southwest. Future efforts targeting orchards in the southwest should focus on conservation for all unique genotypes as a means to sustain both cultural heritage and biological genetic diversity.

The late 19th century is often referred to as the golden years of apple growing in the United States (Calhoun, 1995; Hensley, 2005). Farmstead and kitchen orchards were planted with a wide variety of fruit trees to suit diverse family needs. Historically, rural livelihoods were maintained by growing apples and pears that ripened in summer, would “keep” all winter in the cold cellar, produce

desirable ciders, and those that were amenable to cooking and baking. This period of American horticultural history was preceded by an era of fruit diversification, which spanned much of the 1700s and into the early 1800s. Thousands of trees emerged from seedling orchards, planted for cider and for animal feed, that bore high-quality fruits worthy of naming, conserving, and distributing. These trees were clonally propagated from cuttings and traded and sold to become elements of the diverse orchards of the 19th century (Beach, 1905; Hedrick, 1950). The last remnants of 19th century plantings may still be alive in American landscapes and are the subject of this regional southwestern survey.

USDA pomologist W.H. Ragan undertook the task of recording the names and characteristics of every apple cultivar grown in the United States during the 19th century. In his book, *The Nomenclature of the Apple* (Ragan, 1905), Ragan lists 6654 unique named apple varieties that he found referenced in U.S. literature between the years 1804 and 1904. In 1980, Dan Bussey began expanding on Ragan's register to update descriptions of known cultivars and include additional apple cultivars referenced in the U.S. literature up to the year 1980. Over a decade after beginning this project, Bussey (in press) is close to releasing *The Apple in North America*, which lists over 14,000

named apple cultivars introduced to or selected in North America.

Modern commercial apple production requires consistency of ripening time, quality retention during processing and shipping, and long storage life, and not all varieties can meet these criteria (Goland and Bauer, 2004). Market pressures have reduced the diversity of fruit trees once grown in small family orchards—where diversity of ripening time, sizes, textures, and flavors were celebrated—to only a few handfuls of commonly planted commercial cultivars. Currently, 11 apple cultivars account for over 90% of the apples sold in the United States, with ‘Red Delicious’ constituting 41% of this figure (Dennis, 2008). In *The Fruit, Berry and Nut Inventory* (Whealy, 2001) Kent Whealy lists ≈1500 apple varieties currently available through U.S. nurseries, many of which have been developed through modern fruit breeding. This suggests a substantial decrease in the number of apple cultivars offered through U.S. nurseries over the past century (77% by Ragan's calculations and 89% by Bussey's), although we do not know to what extent this naming actually represented genetically distinct cultivars.

Although the loss of on-farm diversity can be lasting, fruit trees have an advantage over annual crops because these trees can live to remarkably old ages, surviving some fads in consumer demand. Single apple trees have been known to live 150 years or longer. In many areas, it is still possible to find trees of “heirloom” cultivars once abundant at the beginning of the 20th century. Remnant orchards planted before the “modern era” of fruit production (Jackson, 2003) hang on tenaciously around abandoned farmsteads and historic orchards. Although farmstead trees often persist without their original names being retained, they represent a snapshot of the diversity of fruit varieties available over a century ago during the peak of fruit tree diversification.

Morphological and taxonomic traits typically used to differentiate between apple cultivars can be ambiguous as a result of the broad phenotypic variation under different environmental influences. Furthermore, many 19th century apple cultivars are morphologically similar to one another and accurate descriptions are often lacking, making conventional identification methods difficult, if not impossible, for these century-old trees.

Genetic fingerprinting, including microsatellites, have become powerful and accurate tools for analyzing genetic diversity (Hammer et al., 2003). Microsatellite loci, or simple sequence repeats, are short nucleotide sequences of up to six basepairs repeated in tandem, head to tail, without interruption. They are highly polymorphic, codominant markers that have been detected in every organism thus far studied (Hancock, 1999). Gianfranceschi et al. (1998), Hokanson et al. (1998), and Hemmat et al. (2003) were instrumental in developing *Malus*-specific microsatellites in the United States. In Europe, Silfverberg-Dilworth et al. (2006)

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and the High-Quality Disease Resistant Apples for Sustainable Agriculture have been forefront in developing microsatellites for *Malus*. In a similar study in Spain, Pereira-Lorenzo et al. (2007) evaluated the genetic diversity of 114 Spanish apple cultivars comparing the local Spanish landrace genotypes with 26 commercial apple cultivars found in the region. Similar to this study, Pereira-Lorenzo et al. (2007) found high levels of genetic diversity in apple trees in northern Spain based on observed heterozygosity.

In this study, we sampled tissues from 280 apple trees from 43 historic sites within Arizona, Utah, and New Mexico to assess their diversity. Genotypes identified using seven highly variable markers were compared with reference genotypes of known cultivars. The term "historic" is used in this text to refer to farmstead and orchard sites planted during the late 19th and early 20th centuries, and the term "heirloom" refers to cultivars introduced during the 19th century and before as opposed to recent introductions developed through modern fruit breeding programs.

### Materials and Methods

**Field collections.** We collected leaf samples from 280 apple trees located in 43 historic orchard sites on public and private (but not tribal) lands in Arizona, New Mexico, and Utah (Fig. 1). Sampling took place from June through September of 2007. We targeted places with presumed historic orchards and trees of visually differing morphologies. Local experts provided site location information. Sampling permission was obtained and samples were used only for this study, not for gene banking or crop improvement.

We focused sampling efforts primarily on historic farmstead and orchard sites dating back to the 1930s and earlier with priority given to trees planted before 1920. For a handful of the orchard sites such as Capitol Reef National Park, UT, and Slide Rock State Park, AZ, orchard planting dates could be found in historical documentation. Most of the orchard sites lacked written documentation, however, and we had to rely on oral history or visual determination of tree size and locality for approximate ages. We avoided sampling from seedling trees and rootstock trees where it appeared the original grafted top had died. The dry southwest climate limits establishment of naturalized seedling apple trees and many farmstead orchards in the southwest date to the late 19th to early 20th centuries, a time when few seedling orchards were planted in the United States. Leaf samples were collected only from trees that were visibly different from other trees at the same site to avoid repeat sampling of cultivars. However, this was not always possible for trees without fruit.

Global positioning system locations for the sample trees (Garmin eTrex-Vista handheld unit; Garmin, Olathe, KS) in UTM (Nad1983) coordinates were recorded, and small alumi-

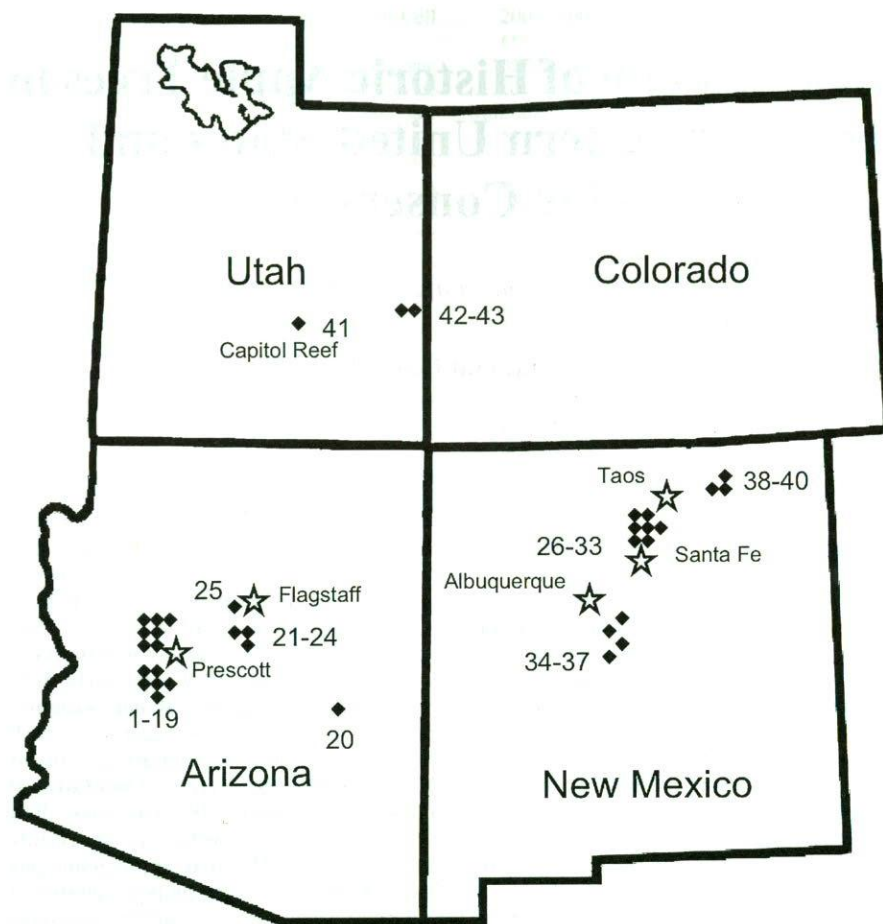


Fig. 1. Historic apple collection sites (numbered and noted by diamonds) in Utah, Arizona, and New Mexico were sampled. Cities are provided for reference purposes (star designation).

num tags were nailed to the trees with a numerical identifier. For each sample,  $\approx 50$  mg of fresh leaf tissue was placed into a 96-well plate and frozen at  $-20^{\circ}\text{C}$  until extraction. Known cultivars were obtained from the USDA-ARS-Plant Genetic Resources Unit, Geneva, NY (PGRU). Varieties that were not available through the Geneva facility were obtained from Lee Calhoun of Calhoun's Nursery in Pittsboro, NC; Ram Fishman of Greenmantle Nursery in Garberville, CA; and Gordon Tooley of Tooley's Trees in Truchas, NM. Leaf samples were processed in the same manner as the unknown samples.

**Microsatellite analysis.** The genetic analysis of the 280 samples and 109 cultivars was performed following procedures described in Volk et al. (2005). We extracted genomic DNA from the leaf samples using Qiagen DNeasy 96 plant kits (Qiagen, Valencia, CA). Two separate sets of DNA were extracted from each sample and run independently. *Malus*-specific microsatellites were amplified using unlinked primers (GD12, GD15, GD96, GD100, GD142, GD147, and GD162) as described by Hokanson et al. (1998) and by Hemmat et al. (2003). Forward primers were labeled with either IRD700 or IRD800 infrared fluorescent dyes (MWG-Biotech, High Point, NC). Unlabeled reverse primers were obtained from IDT (Coralville, IA).

Polymerase chain reactions (PCRs) were carried out in 15  $\mu\text{L}$  total volume. Each 15  $\mu\text{L}$  reaction contained: 0.3  $\mu\text{L}$  GoTaq<sup>®</sup> Flexi Taq Polymerase (Promega, Madison, WI; 5 units/ $\mu\text{L}$ ), 3  $\mu\text{L}$  Promega 5 $\times$  Colorless GoTaq<sup>®</sup> Flexi Buffer (10 mM Tris-HCl, 50 mM KCl, and 0.5% Triton X-100), 1.5  $\mu\text{L}$  of 0.25 mM  $\text{MgCl}_2$ , and 1.5  $\mu\text{L}$  of 0.25 mM dNTPs. Forward and reverse primers were added to a final concentration of 0.25 pM/reaction except for GD12 at 0.3 pM/reaction and GD100 at 0.5 pM/reaction.

Genomic DNA, isolated as described previously, was added at 0.5 ng to 5 ng/reaction. Reaction volumes were adjusted to 15  $\mu\text{L}$  using sterile distilled  $\text{H}_2\text{O}$ . PCR reactions were multiplexed with the following primer sets: GD12, GD100; GD142, GD147, GD162; and GD15, GD96 run together. PCR was carried out using MJ Research PTC 200 Thermocycler (Reno, NV). Amplifications were done using touchdown PCR, in which the thermocycler reduced the annealing temperature  $1^{\circ}$  every cycle, starting at  $63^{\circ}\text{C}$  and ending at  $54^{\circ}\text{C}$ , followed by an annealing temperature of  $55^{\circ}\text{C}$  for 18 cycles and ending with a 2 min  $72^{\circ}\text{C}$  extension.

PCR products were diluted 1:1 with a loading buffer of formamide bromophenol blue loading buffer and were denatured at  $95^{\circ}\text{C}$  for 5 min. Denatured products were diluted 1:10 with additional loading buffer.

Table 1. Ploidy, origin, and probable date of release are provided for 109 apple cultivars introduced to the southwestern United States.<sup>z</sup>

Cultivar name	Ploidy	Origin	Date	Southwest introduction <sup>y</sup>	Reference <sup>x</sup>
Akin	2x	Illinois	1868	Stark 1912	Beach
Albamarle Pippin	2x	Long Island	1759	Stark 1912	Beach
American Summer Pearmain	2x	USA	1817	Stark 1912	Smith
Antanovka	2x	Russia	1826	Texas	Smith
<b>Arkansas Black</b>	2x	Arkansas	1870	Arizona, New Mexico, Texas	Beach
Baldwin	3x	Massachusetts	1784	Arizona, Texas	Smith
Bank's Gravenstein	2x	Nova Scotia	1880	Stark 1912	Whealy
Bedfordshire Foundling	2x	England	1800	unk. <sup>w</sup>	Smith
<b>Ben Davis</b>	2x	Southern U.S.	Early 1800s	Arizona, New Mexico, Texas	Smith
<b>Benoni</b>	2x	Massachusetts	1830	Arizona, New Mexico	Smith
Black Amish	2x	USA, Pennsylvania(?)	unk.	unk.	Calhoun
Black Ben Davis (Gano)	2x	Missouri	1880	TX	Smith
Black Oxford	2x	Maine	1790	unk.	Smith
Boiken	2x	Germany	1828	Stark 1912	Smith
Bonum (Magnum Bonum)	2x	North Carolina	1854	Stark 1912	Smith
Buckingham	3x	Southern U.S.	<1817	New Mexico, Texas	Smith
Campfield	2x	New Jersey	<1817	unk.	Smith
Canada Red	2x	New England	1822	Stark 1912	Beach
Carter's Blue	2x	Alabama	1869	New Mexico, Texas	Smith
Charlamoff (Duchess of Oldenburg)	2x	Russia	1700s	New Mexico, Texas	Smith
Chenango Strawberry	2x	New York or Connecticut	1850	New Mexico	Smith
Chestnut Crab	2x	Minnesota	1946	unk.	Whealy
Cox's Orange	2x	England	1850	Stark 1912	Smith
<b>Delicious</b>	2x	Iowa	1895	Stark 1912	Smith
Dolgo Crab	2x	Russia	<1897	unk.	Whealy
<b>Duchess</b>	2x	Scotland?	<1883	Stark 1912	Smith
<b>Early Harvest</b>	2x	USA	1800s	Arizona, New Mexico, Texas	Smith
Early Strawberry	2x	New York	1838	Arizona	Smith
Esopus Spitzenburg	2x	New York	<1790	Arizona	Smith
Fall Cheese	2x	Virginia	<1905	Stark 1912	Ragan
Fall Pippin	3x	USA	1806	Arizona, Texas	Smith
<b>Fameuse</b>	2x	Canada	1730	Arizona, New Mexico	Smith
Golden Delicious	2x	West Virginia	1890	unk.	Smith
Golden Russet	2x	England	Mid-1700s	Arizona	Smith
Granny Smith	2x	Australia	1868	unk.	Smith
<b>Gravenstein Washington Red</b>	3x	Italy or Germany	1600s	Arizona, Texas	Smith
<b>Grimes Golden</b>	2x	West Virginia	1804	Arizona, New Mexico	Smith
Henry Clay	2x	USA	<1905	Stark 1912	Ragan
Hubardston Nonsuch	2x	Massachusetts	1832	Stark 1912	Smith
Ingram	2x	Missouri	1844	Stark 1912	Smith
Irish Peach	2x	Ireland	<1820	unk.	Smith
Jefferis	2x	Pennsylvania	1830	Texas	Smith
<b>Jonathan</b>	2x	New York	1826	Arizona, New Mexico, Texas	Smith
Keswick Codlin	2x	England	1793	Arizona	Smith
Kinnaird's Choice	2x	Tennessee	Mid-1800s	Texas	Whealy
Knobbed Russet	2x	England	1820	unk.	Smith
Lady (Api)	2x	France	<1628	Stark 1912	Smith
<b>Lawver</b>	2x	Kansas	1868	New Mexico, Texas	Smith
<b>Liveland (Lowland) Raspberry</b>	2x	Russia	<1870	Stark 1912	Smith
Longfield (Glogerovka)	2x	Lithuania or Russia	<1903	Texas	Smith
Lowry	2x	Virginia	1850	Stark 1912	Whealy
Lyman's (Lyman's Large Summer)	3x	Connecticut	1867	unk.	Smith
<b>Maiden Blush</b>	2x	New Jersey	<1817	New Mexico, Texas	Smith
Martha	2x	Wisconsin	<1905	Arizona, New Mexico	Ragan
<b>McIntosh Summerland Red</b>	2x	Canada	1796	Stark 1912	Smith
Mother	2x	Massachusetts	1844	Stark 1912	Smith
<b>Northern Spy</b>	2x	New York	1800	Arizona, New Mexico	Smith
<b>Northwest Greening</b>	2x	Wisconsin	1872	Stark 1912	Whealy
Ohio Nonpareil	2x	Ohio	1848	Stark 1912	Smith
Opalescent	2x	Ohio	1899	Stark 1912	Smith
Ortley	2x	New Jersey	<1817	Arizona, New Mexico, Texas	Smith
<b>Paragon</b>	3x	Tennessee	1830	Arizona	Beach
Peck Pleasant	2x	Rhode Island	1832	Arizona	Smith
Pewaukee	2x	Wisconsin	1870	New Mexico	Smith
Pitmaston Pineapple	2x	England	1785	unk.	Smith
Porter	2x	Massachusetts	1800	New Mexico	Smith
Primate	2x	New York	1840	Stark 1912	Smith
<b>Red Astrachan</b>	2x	Russia	<1816	New Mexico	Smith
Red Bietigheimer (Roter Stettiner)	3x	Germany	1598	Arizona, New Mexico, Texas	Smith
<b>Red June (Carolina Red June)</b>	2x	North Carolina	1848	New Mexico, Texas	Smith
<b>Red Ralls (Ralls Janet)</b>	2x	Virginia	1800	Arizona, New Mexico	Smith
<b>Rhode Island Greening</b>	3x	Rhode Island	1650	Arizona, New Mexico	Smith
Ribston	3x	England	1707	Arizona	Smith
Roman Stem	2x	New Jersey	1817	Stark 1912	Smith

(Continued on next page)

Table 1. (Continued) Ploidy, origin, and probable date of release are provided for 109 apple cultivars introduced to the southwestern United States.<sup>2</sup>

Cultivar name	Ploidy	Origin	Date	Southwest introduction <sup>2</sup>	Reference <sup>3</sup>
<b>Rome Beauty law</b>	2x	Ohio	1848	Arizona, New Mexico, Texas	Smith
Rosemary Russet	2x	England	1831	unk.	Smith
Roxbury Russet	3x	Massachusetts	1649	Arizona	Beach
Shockley	2x	Georgia	1854	New Mexico, Texas	Smith
Smith's Cider	2x	Pennsylvania	<1817	Arizona, Texas	Smith
Sops of Wine	2x	England	1831	New Mexico	Smith
Stark	3x	Ohio	1867	Arizona	Smith
<b>Stayman</b>	3x	Kansas	1875	Stark 1912	Smith
Summer Champion	2x	Arkansas	1897?	Stark 1912	Calhoun
Swaar	2x	New York	1804	Arizona, New Mexico	Smith
Sweet Bough (Bough)	2x	USA	1817	Arizona, Texas	Smith
Sweet Dixon	2x	North Carolina	<1905	New Mexico, Texas	Ragan
<b>Tolman Sweet</b>	2x	USA	1822	Arizona, New Mexico	Smith
Tompkin's King	3x	New Jersey	1804	Stark 1912	Smith
<b>Transcendent Crab</b>	3x	unk.	<1844	New Mexico	Bussey
Twenty Ounce Pippin	2x	Connecticut or New York	1844	Texas	Smith
Virginia Crab (Hewes)	2x	Virginia	<1803	unk.	Bussey
Virginia Beauty	2x	Virginia	1826	Stark 1912	Whealy
Vixin Crab	3x	unk.	unk.	unk.	unk.
<b>Wagner</b>	2x	New York	1791	Stark 1912	Smith
<b>Wealthy</b>	2x	Minnesota	1860	New Mexico, Texas	Smith
<b>Westfield Seek No Further</b>	2x	Massachusetts	1796	unk.	Smith
<b>White Astrachan</b>	3x	Sweden or Russia	1748	Arizona	Smith
White Winter Pearmain	2x	USA	1849	Stark 1912	Smith
Wickson	2x	California	1944	unk.	Whealy
<b>Winesap</b>	2x	New Jersey	<1817	Arizona, New Mexico, Texas	Smith
<b>Winter Banana</b>	2x	Indiana	1876	unk.	Smith
Winthrop Greening	2x	Maine	1800	unk.	Smith
<b>Wolf River</b>	2x	Wisconsin	1875	New Mexico	Smith
Yarlington Mill	2x	England	unk.	unk.	Whealy
Yates	2x	Georgia	1813	Stark 1912	Smith
<b>Yellow Bellflower</b>	2x	New Jersey	<1817	Arizona, Texas	Smith
Yellow Newtown Pippin <sup>2</sup>	2x	Long Island	1759	unk.	Beach
<b>Yellow (White) Transparent</b>	2x	Russia or Baltic States	1800s	Texas	Smith
York Imperial	2x	Pennsylvania	1830	Arizona, New Mexico	Smith

<sup>2</sup>Cultivars identified in bold were identified in historic farmsteads.

<sup>3</sup>Reference samples were based off of southwest introductions by state agriculture experiment stations (Arizona, New Mexico, Texas), 1912 Stark BO's catalog entries, if known.

<sup>4</sup>Origins and probable date of release are based on Beach (1905), Bussey (in press), Calhoun (1995), Ragan (1905), Smith (1971), and Whealy (2001).

<sup>5</sup>Unknown.

Diluted products were loaded on gels (6.5% KB Plus acrylamide; LI-COR, Lincoln, NE) and run in 1× TBE buffer (89 mM Tris, 89 mM boric acid, and 20 mM EDTA) for 1 h, 45 min at 1500 V, 40 W, 40 mA, and 45 °C in a LI-COR 4200 DNA Sequencer. Digital images of the gels collected by LI-COR Saga Generation2 software were manually analyzed using Saga software. Each allele at each locus was manually scored in Saga before being compared with the duplicate sample.

Ploidy was determined using flow cytometry by Gerard Geenen of the Plant Cytometry Services, Schijndel, The Netherlands.

**Microsatellite data analysis.** Genotypes for the 280 samples and the 109 cultivars were compared manually in Microsoft Excel 2004 for Mac, Version 11.3.7 (Microsoft, Redmond, WA). Allele frequencies and observed and expected heterozygosities were computed using GenAlEx version 6 (Peakall and Smouse, 2006). Principal component analysis (PCA) ordinations were performed using PC-ORD version 4.0 (MjM Software Design, Gleneden Beach, OR).

## Results and Discussion

We found considerable genetic diversity in historic southwest orchard and farmstead sites. The "unknown" apple trees were com-

pared with 109 known cultivars introduced into the southwest in the late 19th century by USDA agricultural experiment stations and by Stark Brother's Nursery, the largest mail order nursery during the late 19th and early 20th centuries. DNA fingerprints revealed that 120 of the 280 sample trees were indistinguishable from the fingerprints of 34 cultivars (Tables 1 and 2). The remaining 160 historic tree samples did not match any of the reference cultivars. These 160 samples represented 110 unique genotypes. These unknown genotypes could be regionally unique cultivars, local seedlings, cultivars extinct from the nursery trade, or extant cultivars originally from other regions that have not been recorded as being introduced into the southwest. For two samples, the duplicate genotypes did not match, suggesting different DNA source material. This may have been a result of leaves being collected from vegetative rootstock and from the grafted tree or a result of human error. Both samples were discarded from the study. In total, the 280 historic trees represented 144 distinct genotypes.

Only five of the 34 identified cultivars appeared to be commonly distributed. 'Ben Davis', 'Delicious', 'Grimes Golden', 'Jonathan', and 'Winesap' each represented more than four trees in the study found at multiple

locations. A number of trees matched genotypes of named cultivars for all but one or two alleles at the locus GD100 (Table 2). It is not known if these 1- or 2-bp shifts represent morphologically different varieties or were a result of error during allele scoring.

The seven microsatellites were sufficient to differentiate between most samples and cultivars in this study. However, several of the cultivars had identical fingerprints. This suggests that these cultivar names are synonyms of the same cultivar, are close sport mutations not differentiable by these microsatellites (see Hokanson et al., 1998), or are mislabeled at their source nursery or genebank location. Named sets of cultivars Albarle Pippin and Yellow Newtown Pippin, Early Strawberry and Yates, Fameuse and Canada, and Maiden Blush and Chenango Strawberry were indistinguishable from each other.

Ploidy results revealed 24 of the 280 samples (8.6%) were triploid (3x = 51), whereas the remaining 91.4% were diploid (2x = 34). Triploids arise spontaneously in 2x-by-2x crosses and typically have larger fruit than diploid apple trees (Ferree and Warrington, 2003). Based on field observations, many of the triploids in this study appear to be late-ripening, large-fruited winter apples.

Table 2. Identification of cultivars identified in historic farmsteads in the southwestern United States.

Cultivar	Trees identified (no.)	Land ownership <sup>a</sup>	Orchard type <sup>b</sup>	Present condition <sup>c</sup>	Nursery sources <sup>d</sup> (no.)	Use
Arkansas Black	3	AZSPS, private	Commercial and farm	Maintained	35	Cider, fresh eating, and cooking
Ben Davis	16	NFS and private	Commercial and farm	Abandoned	10	Long storage life
Benoni	2	Private	Commercial	Abandoned	6	Dessert
Delicious	12	NFS and private	Commercial and farm	Maintained	36	Fresh eating and dessert
Duchess	1	Private	Farm	Maintained	17	Cooking, poor storage life
Early harvest <sup>e</sup>	1	Private	Farm	Abandoned	18	Cooking
Fameuse	2	Private	Farm	Abandoned	19	Cider, fresh eating, and cooking
Gravenstien, WA Red	1	Private	Farm	Abandoned	31	Cooking and cider
Grimes Golden <sup>e</sup>	6	NPS and private	Commercial and farm	Maintained	35	Cider and dessert
Jonathan	17	NFS and private	Commercial and farm	Maintained	44	Cooking and fresh eating
Lawver <sup>e</sup>	1	Private	Commercial	Abandoned	1	Fresh eating
Liveland Raspberry	1	NFS	Farm	Abandoned	11	Fresh eating
Maiden Blush	2	Private	Commercial	Abandoned	16	Cider, fresh eating, cooking
McIntosh Summerland	1	Private	Farm	Abandoned	46	Cider, fresh eating, cooking
Northern Spy	1	Private	Commercial	Abandoned	39	Cider, fresh eating, and cooking
Northwest Greening	2	Private	Farm	Maintained	15	Winter cooking apple
Paragon	2	AZSPS and private	Farm	Maintained	1	Winter cooking apple
Red Astrachan <sup>e</sup>	2	NFS and private	Commercial and farm	Abandoned	18	Fresh eating and cooking
Red June	1	NFS	Farm	Abandoned	19	Cider, fresh eating, and cooking
Red Ralls	3	Private	Farm	Maintained	11	Fresh eating
Rhode Island Greening <sup>e</sup>	3	NPS and private	Commercial and farm	Maintained	12	Winter cooking apple
Rome Beauty Law	2	Private	Farm	Abandoned	11	Cooking and cider
Stayman	3	Private	Farm	Maintained	24	Winter cooking and cider apple
Tolman Sweet <sup>e</sup>	2	Private	Commercial and farm	Abandoned	17	Cider, fresh eating, and cooking
Transcendent Crab	1	NFS	Farm	Abandoned	2	Cooking
Wagner	2	NFS	Farm	Abandoned	8	Cider, fresh eating, and cooking
Wealthy <sup>e</sup>	1	NFS	Farm	Abandoned	24	Cider, fresh eating, and cooking
Westfield Seek No Further <sup>e</sup>	4	NFS	Farm	Abandoned	16	Dessert
White Astrachan <sup>e</sup>	3	NFS and private	Commercial and farm	Abandoned	2	Fresh eating and dessert
Winesap	13	Private	Commercial and farm	Abandoned	23	Cider, fresh eating, and cooking
Winter Banana	1	NFS	Farm	Abandoned	27	Cider
Wolf River	4	AZSPS and private	Commercial and farm	Maintained	31	Cooking
Yellow Bellflower	3	NFS	Farm	Abandoned	16	Winter cooking apple
Yellow Transparent	1	Private	Farm	Abandoned	36	Fresh eating and cooking

<sup>a</sup>Tree(s) were located on private property, National Forest Service land (NFS), National Park Service land (NPS), or Arizona State Park land (AZPS).

<sup>b</sup>Identifies if tree is from a commercial orchard or farmstead orchard.

<sup>c</sup>Indication if current tree locations are maintained orchards or abandoned.

<sup>d</sup>The number of U.S. nurseries that still offered the cultivar in 2000 of 277 surveyed (Whealy, 2001).

<sup>e</sup>Cultivars identical to the reference cultivar except at marker GD100 where there was a 1-or 2-bp difference.

High levels of observed heterozygosity (0.92 for GD142, 0.90 for GD162, and 0.88 for GD147 and GD96) in the samples suggest that relatively high levels of genetic diversity are represented (Table 3). Heterozygosity was calculated from a sample size of 144 individuals, representing each of the 144 distinct genotypes found growing in the southwest. Observed heterozygosity was calculated by dividing the number of heterozygotes at a locus by the number of individuals surveyed. Expected heterozygosity assumes Hardy-Weinberg equilibrium but is included as a reference. The multiplicative probability of a multilocus genotype determines the power of discrimination; the high heterozygosity of the sampled loci therefore makes the probability of distinctly different genotypes being identical at all seven microsatellite loci very slight. Microsatellite GD100 had 6% missing data as a result of poor amplification during PCR for several samples. We chose not to score this allele in these cases to avoid potential scoring error. Heterozygosity and allele frequencies should be interpreted with dubiety for this allele.

PCA was performed on the samples to visualize the genetic difference between apple genotypes (Fig. 2). In the PCA ordination, the two distinct clusters appear to be the

Table 3. Sample size, number of alleles, observed heterozygosity, and expected heterozygosity were calculated for samples with unique genotypes.

Microsatellite marker	Sample size number	Number of alleles	Observed heterozygosity	Expected heterozygosity
GD12	144	11	0.833	0.751
GD15	144	2	0.021	0.021
GD96	144	14	0.875	0.853
GD100	134	9	0.485	0.814
GD142	144	13	0.924	0.864
GD147	144	12	0.868	0.817
GD162	144	13	0.896	0.827

result of the presence of two larger alleles at marker GD12 (182 and 190 bps) instead of the more common 148- to 162-bp length alleles. Such clustering could be indicative of a shared genetic heritage between genotypes in the upper left cluster, although there does not appear to be any morphological association with the groups.

Unknown genotypes were labeled in the PCA with a state prefix attached to the end of the sample number to show possible associations of geographic origin to genotype. Geographic separation of samples would indicate that different sources of apple trees were grown in the various regions. Spanish priests, explorers, and settlers introduced apple trees to New Mexico as early as the 17th century

(Dunmire, 2004) as did the Archbishop Lamy of Santa Fe in the 19th century (Horgan, 1975). Geographic differentiation could also imply different apple preferences associated with different regions. However, there was no apparent genetic separation by geographic origin among samples. The historic trees in the southwest might all share the same recent parents, or the parent diversity could have been obscured by small sample size. Efforts were made to avoid seedling apple trees during sampling. As mentioned in the "Materials and Methods" of this article, we focused sampling efforts on grafted as opposed to seedling trees; however, a small percentage of the samples are likely to be of seedling origin and not named cultivars. Seedling trees

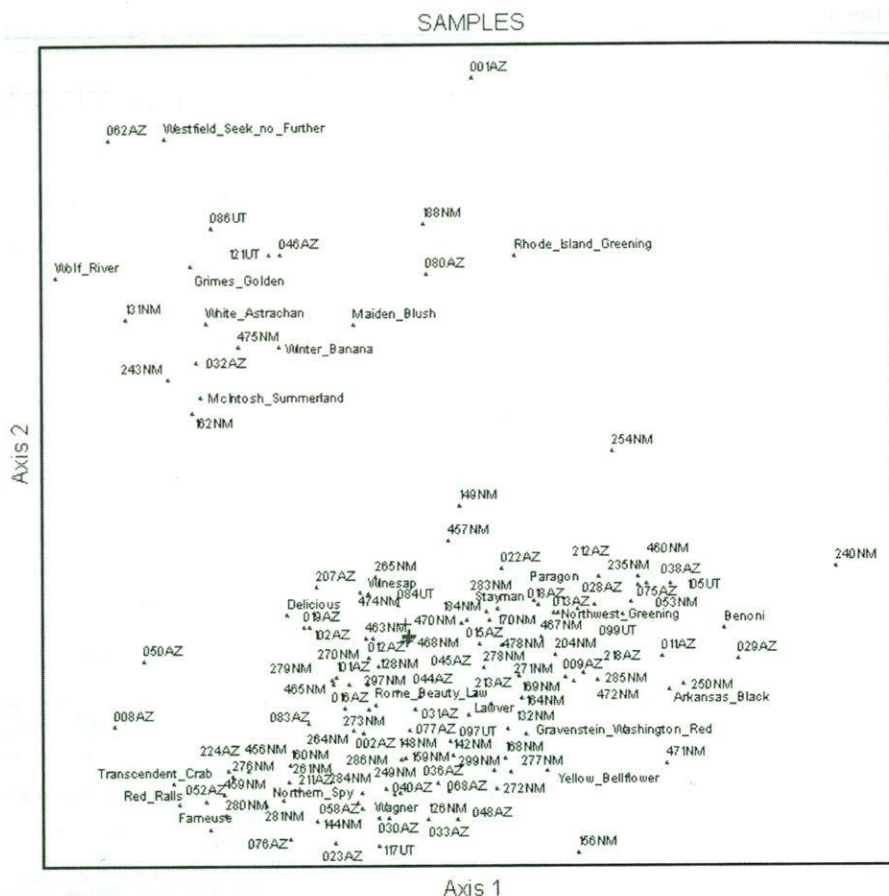


Fig. 2. Principal component analysis of unique apple genotypes. Sample numbers and state of origin are provided for unknown varieties and names are provided for known varieties.

would not share the same “heirloom” status as named 19th century cultivars but may still possess useful traits or local adaptations.

For this study, we fingerprinted cultivars from the USDA National Germplasm Collection and from private nurseries to compare with the unknown samples. We restricted the number of reference cultivars to 109 likely introduced into the southwest. The existence of a DNA fingerprint database for correctly identified fruit tree cultivars such as those at the PGRU would allow studies such as this one to answer more questions about the identities of the many unknown apple trees growing on abandoned farms and in parks and forests across the country.

Apple plantings in a number of orchards and farmsteads cultivated in the 19th and early 20th centuries in the U.S. southwest still survive, although many have been abandoned. From 43 historic sites, 280 apple trees were sampled and compared with 109 cultivars at the PGRU and in private nurseries using microsatellite analysis. The 280 samples yielded 34 named cultivars and 110 unique genotypes. These results suggest that the historic orchards in the southwest had a high diversity of genotypes. Additional genetic fingerprinting of apple cultivars in the USDA

PGRU will potentially enable us to identify the unknowns in this survey. Until such work is undertaken, these unknown genotypes should be conserved and analyzed for useful traits.

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